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| (54) Title: METHODS FOR SELECTIVELY EXPANDING STEM CELLS (57) Abstract The present invention provides a method for selectively expanding stem cells, comprising the steps of (a) separating stem cells from other cells, and (b) incubating the separated stem cells in a selected medium, such that the stem cells are selectively expanded. | | |

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Description

METHODS FOR SELECTIVELY EXPANDING STEM CELLS

5

Cross-Reference to Related Application

This application is a continuation-in-part of pending U.S. Application Serial No. 07/513,543, which was filed April 23, 1990.

10

Technical Field

The present invention relates generally to cells which make up bone marrow, and more specifically, to devices and methods which may be utilized to selectively expand the number of stem cells.

15

Background of the Invention

Cancer accounts for over one-fifth of the total mortality in the United States, and is the second leading cause of death. The leading types of cancer in men are lung, prostate, and colorectal cancer, and for women, breast, lung and colorectal cancer. Currently, most cancers are treated with a combination of therapies involving surgery, and chemotherapy and/or radiation therapy.

One difficulty with chemotherapy and radiotherapy, however, is that it also destroys an individual's immune system, as well as stem cells, the progenitor cells of the immune system. In order to reconstitute the immune system, a patient undergoes bone marrow transplantation with generally either allogenic or autologous bone marrow. Many individuals, however, die because their own bone marrow is involved with the cancer, or a histocompatible donor cannot be found.

One method that has been suggested to overcome this difficulty is the use of long-term marrow cultures which prolong the life of engrafting cells. For example, Dexter *et al.* (*J. Cell. Phys.* 91:335, 1976) describes conditions for proliferating stem cells *in vitro* (hereinafter referred to as "Dexter cultures"). Briefly, this method involves the establishment of an adherent monolayer of stromal cells, which supports the viability of stem and early progenitor cells. Dexter cultures, however, are ultimately disadvantageous because they only slow the death rate of stem and progenitor cells, and do not provide the desired result of increasing the number of such cells.

The present invention provides devices and methods for selectively expanding stem cells. These devices and methods overcome disadvantages of prior devices and methods, and further provide other related advantages.

5 Summary of the Invention

Briefly stated, the present invention is directed toward devices and methods for selectively expanding stem cells, and for obtaining mature hematopoietic cells. Within one aspect of the present invention, a method for selectively expanding stem cells is provided, comprising the steps of (a) separating
10 stem cells from other cells, and (b) incubating the separated stem cells in a selected medium, such that the stem cells are selectively expanded. Within another aspect of the invention, a method for selectively expanding stem cells is provided, comprising the steps of (a) periodically separating stem cells from mature cells, and (b) incubating the separated stem cells in a selected medium
15 such that the stem cells are selectively expanded. Within various embodiments, the selected medium contains Stem Cell Growth Factor, Interleukin-3, Granulocyte-Macrophage Colony-Stimulating Factor, Granulocyte Colony-Stimulating Factor, Interleukin-6, or Mast Cell Growth Factor.

Within other embodiments, the stem cells are separated on an
20 affinity column, or by Flow Cytometry. Within further embodiments, the separated stem cells are incubated in a petri dish, in a sterile bag, or in hollow fibers.

Within another embodiment of the present invention, a method for selectively expanding stem cells is provided, comprising the steps of (a) separating
25 stem cells from other cells on an affinity column, and (b) incubating the separated stem cells in a sterile bag with a medium containing Stem Cell Growth Factor, Interleukin-3, and Granulocyte-Macrophage Colony-Stimulating Factor.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached
30 drawings.

Brief Description of the Drawings

Figure 1 is a graph which illustrates the effect of separating out stem cells on stem cell expansion, as determined by the increase in total cell number.

35 Figure 2 is a graph which illustrates the effect of separating out stem cells on stem cell expansion, as determined by the increase in CFCs.

Figure 3 is a graph which illustrates the effect of various growth factors on total cell number.

Figure 4 is a graph which illustrates the effect of various growth factors on the number of CFCs.

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Detailed Description of the Invention

The present invention provides devices and methods for selectively expanding stem cells. Within the context of the present invention, the term "stem cell" refers to totipotent hematopoietic stem cells as well as early progenitor cells such as colony-forming cells (CFCs). These cells may be differentiated from other cells based upon the presence of CD 34 receptors.

As noted above, within one aspect of the present invention, methods are provided for selectively expanding stem cells, comprising the steps of (a) separating stem cells from other cells, and (b) incubating the separated stem cells with a selected media which is capable of expanding the stem cells.

Utilizing the devices and methods which are described in greater detail below, stem cells may be separated from various blood products, including for example, peripheral blood and whole bone marrow. For purposes of the present invention, stem cells are considered to be separated if at least 20% of the separated cells are CD 34 positive cells. Preferably, the CD 34 positive cells should be separated to provide greater than 90% purity. In addition, it may be desirable to keep the total numbers of platelets, granulocytes, and red cells as low as possible in order to prevent clumping and the release of degradative enzymes which decrease engrafting cell recovery and viability. More specifically, it may be desirable that the stem cells contain less than about 1% platelets, less than 50% and preferably less than about 25% granulocytes, and less than 10% and preferably less than about 1% red cells.

Separation of stem cells may be accomplished through use of a ligand which specifically recognizes antigens on these cells. For example, antibodies which specifically recognize the CD 34 antigen may be utilized in the devices and methods described below in order to separate stem cells. Representative examples of antibodies which specifically recognize the CD 34 antigen include My-10 and HPCA2 (Becton-Dickinson, Mountain View Calif.) and 12.8 (CellPro®, Bothell, Wash.).

Various methods and devices may be utilized to separate stem cells, including the use of magnetic beads, panning, and flow cytometry (Fluorescence Activated Cell Sorting "FACS") (see, for example, U.S. Patent Nos. 4,714,680 and

4,965,204, herein incorporated by reference). Particularly preferred methods and devices are immunoaffinity columns such as those which are described in pending U.S. Application Serial No. 07/513,543, entitled "Immunoselection Device and Method" (hereby incorporated by reference in its entirety). Briefly, this application describes methods and devices for isolating or separating target particles such as stem cells, from a mixture of non-target and target particles. Included within this application is a discussion of devices and methods wherein target particles are separated in a direct method by passing the particles through a column containing a bed of low nonspecific binding porous material which has a ligand capable of specifically binding to the target particles. Within one aspect of the application, a device is provided which generally comprises (a) a column having a proximal end with an inlet port through which fluid may enter the column and a distal end with an outlet port through which fluid may exit the column, (b) a bed of low nonspecific binding porous material within the column, the porous material having a biotin adsorbing group immobilized on the surface thereof, wherein the pores of the porous material are of a size sufficient to allow the biotin adsorbing group to enter into the pores, but not so large as to allow collapse of the bed, and wherein the interstitial spaces of the bed are of a size sufficient to allow the particles to flow through the bed. The device may further comprise a means, located within the column, for agitating the porous material upon the application of an external force, such that bound target particles are released from the porous material. Within other aspects of this application the target particles are separated by either a one-step or two-step method utilizing avidin and biotin. It should also be noted, however, that for purposes of the present invention other materials may be utilized within the immunoaffinity column, including for example non-porous materials.

A particularly preferred immunoaffinity column is described in pending U.S. Application (Attorney's Docket No. 200072.407) entitled "Improved Apparatus and Method for Cell Separation" (hereby incorporated by reference in its entirety). Briefly, within one aspect of this application, a "cell separator" is provided including a column assembly for separating target cells from a sample fluid, the column assembly including a column, a sample fluid supply bag and a fluid collection bag wherein the column is provided for receiving the sample fluid from the sample fluid supply bag and for separating the target cells from the sample fluid and retaining the target cells, and wherein the fluid collection bag is provided for receiving the target cells after being released from the column, said cell separator, comprising an agitation means for agitating the contents of the

column to assist in releasing the sample cells retained in the column, the agitation means being responsive to a drive signal for varying amounts of agitation of the contents of the column to vary the rate at which the sample cells are released, column sensor means for providing a column signal indicative of the optical
5 density of fluid flowing out of the column and into the fluid collection bag, a column valve means response to a column valve control signal for selectively enabling the fluid coming out of the column to flow into the fluid collection bag, and a data processor means for controlling the operation of the cell separator, the data processor means being responsive to the column signal for providing the
10 drive signal and the column valve control signal to prevent inadequate concentrations of the target cells from being collected. One embodiment of this invention is the CEPRATE LC™ cell separation system which is available from CellPro® (Bothell, Wash.).

The separated stem cells are then incubated in a selected medium
15 such that the stem cells are selectively expanded. Within the context of the present invention, stem cells are "selectively expanded" if stem cells increase in number more than other cell types as a whole. Briefly, the media should comprise a nutrient base which maintains cell viability, as well as a factor which expands the number of stem cells. Representative examples of nutrient bases include RPMI,
20 TC 199, or Iscoves DMEM, which have been supplemented with a protein such as fetal bovine serum. Alternatively, the nutrient base may be a defined medium such as Ex Vivo. Various components may be utilized in order to selectively expand stem cells. Representative factors include Interleukin-1, Interleukin-3, Interleukin-4, Interleukin-6, Interleukin-7, SCF, GM-CSF, G-CSF, M-CSF, TGF-
25 β , TNF- α , α -INF, FGF, PDGF, IGF-1, and IGF-2. Particularly preferred factors include Stem Cell Growth Factor (Amgen, Thousand Oaks, Calif.), Interleukin-3, Granulocyte-Macrophage Colony-Stimulating Factor (Immunex, Seattle, Wash.), Granulocyte Colony-Stimulating Factor, Interleukin-6 (Amgen, Thousand Oaks, California), and Mast Cell Growth Factor (Immunex, Seattle, Wash.).

30 One may readily determine whether a medium selectively expands stem cells by purifying stem cells as described above, and incubating them as described below with a selected medium. The number of stem cells and other cells are counted prior and subsequent to incubation. As noted above, stem cells have been selectively expanded if they increase in number, while the other cells do
35 not. Total numbers of cells may be counted by standard techniques (e.g., hemacytometer), and numbers of stem cells may be counted by flow cytometry

(i.e., cells labeled with fluorescently conjugated anti-CD34 antibody may be counted by FACS) or as described below in Example 3.

The separated stem cells may be incubated in a variety of containers. Particularly preferred containers include: petri dishes (Corning Glass Works, Corning, N.Y.), 6-well plates (Costar), gas permeable sterile bags such as Stericell (Terumo, Elkton, Md.), and Lifecell (Baxter, Deerfield, Ill.), and hollow fibers such as CellPhar2 (Unisyn Fibertec, San Diego, Calif.), Accusys (Endotronics, Minneapolis, Minn.), or Vitafiber (Amicon, Danvers, Mass.). Preferably, the cells should be incubated in an atmosphere of 5% to 10% CO₂, and at a temperature of about 37°C.

Within another aspect of the invention, a method is provided for selectively expanding stem cells, comprising the steps of (a) periodically separating stem cells from mature cells, and (b) incubating the separated stem cells in a selected medium such that the stem cells are selectively expanded. Briefly, mature cells (which include not only terminally differentiated blood cells, but cells of an intermediate lineage) are believed to inhibit the expansion and differentiation of stem cells via a feedback control mechanism. Removal of mature cells from a culture thus permits expansion of the stem cells to many times their original numbers. Within the context of the present invention, periodically separating means removal of mature cells at least every 10 days.

Various methods may be utilized in order to periodically separate stem cells. For example, within one embodiment, cells are separated on an affinity column as described above, incubated in a selected medium utilizing any of the containers discussed above, and then subsequently re-separated in order to separate the stem cells from the newly differentiated mature cells.

Within another aspect of the invention, stem cells are continuously separated while being perfused with a selected medium such that the stem cells are selectively expanded. Briefly, within one embodiment this may be accomplished by continuously perfusing the selected media through a separation device (such as an affinity column as discussed above). Stem cells are retained in the separation device while mature cells (which do not have CD 34 antigens) pass through the device. As stem cells grow and expand in number, the new stem cells similarly adhere to the separation device, while newly differentiated cells with no CD 34 antigens pass through the device.

Within another embodiment of the invention, stem cells are continuously separated in a perfusion device by passing beads through the perfusion chamber which have been coated with immobilized ligands to surface

antigens found on mature cells, so that the mature cells are bound and carried out of the perfusion chamber by the beads.

The following examples are offered by way of illustration, and not by way of limitation.

5

EXAMPLES

EXAMPLE 1

Preparation of an Avidinated Biogel

A. CARBOXYLATION OF A POLYACRYLAMIDE GEL

10 Seventeen grams of dry Biogel P-60™ (50-100 mesh (wet), coarse beads) (BIORAD, Catalog No. 150, 1630, Richmond, Calif.) are added to 1.5 l of 0.5 M NaHCO₃/0.5 M Na₂CO₃. The pH is adjusted to 10.5 with NaOH and carefully stirred with a mixer (RZR1, Carfamo, Warton, Ontario, Canada) so as not to damage the beads for approximately 20 to 30 minutes. The mixture is then
15 placed in a 60°C water bath. After the mixture reached a temperature of 60°C, it is incubated for an additional 2 hours (at 60°C) with occasional stirring. The mixture is then removed from the water bath, and placed in an ice bath to bring the mixture temperature down to room temperature.

20 The beads are washed several times with distilled or deionized water, followed by several washings with PBS using a coarse glass filter connected to a vacuum. The carboxylated gel may be stored in PBS at 4°C, and is stable for up to one year if sterilized or stored with a preservative.

B. AVIDIN CONJUGATION OF CARBOXYLATED BIOGEL

25 PBS is first removed from a measured amount of carboxylated Biogel by filtering with a coarse glass filter connected to a vacuum. The gel is then equilibrated in distilled or deionized water for 15 to 30 minutes. Equilibration in water causes an expansion of the gel to a volume of about 4 times its previously measured amount. The gel is resuspended in 10 ml of distilled or
30 deionized water for each ml of gel (as originally measured in PBS).

 Thirty mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC-HCl) (Sigma Chemical Co., Catalog No. E7750, St. Louis, Mo.) is added for each ml of gel as originally measured. The pH is rapidly adjusted to 5.5 by dropwise addition of HCl. Care is taken to maintain the pH at 5.5; pHs of less
35 than 5.0 or greater than 6.0 result in significantly less activation of the Biogel. The mixture is stirred for five minutes.

Avidin (International Enzymes, Inc., Fallbrook, Calif.) is dissolved at a concentration of between 10 and 100 mg/ml in deionized water. Next, 1 mg of avidin is rapidly added for each ml of gel (as originally measured in PBS). The mixture is stirred for 1.5 hours. Next, 2 M glycine is added to give a final
5 concentration of 0.2 M glycine in the mixture, and stirred for an additional 1 hour.

The gel is washed with several volumes of PBS using a coarse glass filter and vacuum, and stored in PBS at 4°C. The gel is stable for approximately one year.

10

EXAMPLE 2

Isolation of Engrafting Cells

A. PREPARING THE BUFFY COAT CELLS

A sample of bone marrow is centrifuged at 240 x g for 15 minutes.
15 The plasma is removed (and is retained for later use), and the remaining buffy coat cells are centrifuged once more at 240 x g for 15 minutes in order to remove red blood cells. The buffy coat cells are washed twice with RPMI by centrifugation at 180 x g for 10 minutes. The cells are then resuspended to a final concentration of 1×10^8 white cells/ml in RPMI plus 1% BSA.

20

B. INCUBATION OF BUFFY COAT CELLS WITH ANTIBODY

The suspension of buffy coat cells is incubated with a final concentration of 20 μ g/ml biotinylated anti-CD 34 antibody (CellPro®, Bothell, Wash.) at room temperature for 25 minutes. The antibody-cell mixture is then
25 washed twice with PBS by centrifugation at 180 x g for 10 minutes. The cells are then resuspended at a concentration of 1×10^8 white cells/ml in PBS.

C. COLUMN OPERATION AND RESULTS

A CEPRATE LC™ (CellPro®, Bothell, Wash.) separating system
30 was utilized essentially according to the manufacturer's instructions. Briefly, the instrument was set up, the tubing connected, reagent's were loaded, and the process run was begun with the antibody treated cells. The cells were pumped through the column, the column was washed with PBS, then the adsorbed cells were released via the magnetically driven impeller. The adsorbed cells were
35 accumulated in a collection bag.

D. RESULTS

Ten billion bone marrow cells were passed through the column; 200 million of the cells were bound to the column and were recovered in the collection bag. Viability of the collected cells was 91% as measured by trypan blue exclusion. The collected cells were 75% CD 34⁺ as measured by FACS analysis.

EXAMPLE 3

Determination of CFC Viability and Recovery

One ml per 35 mm plate of Iscove's Methylcellulose (Terry Fox Laboratories, Vancouver, British Columbia, Canada) supplemented with 2 mM L-glutamine and 50 ng/ml gentamicin was warmed to 37°C. Cells were plated in triplicate at 3-fold dilutions to improve the accuracy of the assay. The highest number of cells plated was 10⁵/plate except for column-purified cells which were plated at 3 x 10³ and less. The cells were spread evenly over the surface of each plate and then incubated in a humidified incubator at 37°C with 5% CO₂ in air for 10 to 14 days. Colonies were counted if they contained more than 50 cells and scored as CFU-GM, BFU-E, or other (e.g., CFU-GEMM). The number of various types of colonies were summed to give the total number of colony-forming cells (CFCs).

EXAMPLE 4

Stem Cell Expansion

A. COMPARISON OF THE EXPANSION OF SEPARATED STEM CELLS VERSUS STEM CELLS IN WHOLE MARROW

Stem Cells which were purified as described above in Example 2 were grown in a solution containing RPMI 1640, 10% Fetal Bovine Serum HYCLONE®, Logan, Utah), 50 ng/ml Stem Cell Growth Factor, 50 ng/ml Interleukin-3, 20 ng/ml Granulocyte-Macrophage Colony-Stimulating Factor, and 20 ng/ml Granulocyte Colony-Stimulating Factor. The cells were plated at 10⁶ per plate in 1 ml of media. On days 7, 14, and 21 cells were removed and replated for CFC assays as described in Example 3. Viable cells were counted by hemacytometer using trypan blue.

As illustrated by total cell count in Figure 1, and the number of CFCs in Figure 2, stem cell separation prior to culturing the cells dramatically improved stem cell growth and expansion.

B. FACTORS WHICH CAUSE STEM CELL EXPANSION

In order to determine what growth factors are useful for expanding stem cells, the following assay was undertaken. Briefly, stem cells which were purified as described above, were plated at a density of 10^6 cells per plate in Corning 35 mm plates. The cells were fed a solution containing RPMI 1640 supplemented with 10% fetal bovine serum, and various combinations of the following growth factors: (1) 50 ng/ml Stem Cell Growth Factor (Amgen, Thousand Oaks, Calif.), (2) 50 ng/ml Interleukin-3, (3) 20 ng/ml Granulocyte-Macrophage Colony-Stimulating Factor (Immunex, Seattle, Wash.), and (4) Granulocyte Colony-Stimulating Factor (Genzyme, Cambridge, Mass.).

As illustrated in Figures 3 and 4, both media containing SCF expanded stem cells (as determined by the increase in CFC number).

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

Claims

1. A method for selectively expanding stem cells, comprising:
 - (a) separating stem cells from other cells; and
 - (b) incubating the separated stem cells in a selected medium, such that the stem cells are selectively expanded.
2. A method for selectively expanding stem cells, comprising:
 - (a) periodically separating stem cells from mature cells; and
 - (b) incubating the separated stem cells in a selected medium such that the stem cells are selectively expanded.
3. The method of claims 1 or 2 wherein said selected medium contains Stem Cell Growth Factor.
4. The method of claims 1 or 2 wherein said selected medium contains Interleukin-3.
5. The method of claims 1 or 2 wherein said selected medium contains Granulocyte-Macrophage Colony-Stimulating Factor.
6. The method of claims 1 or 2 wherein said selected medium contains Granulocyte Colony-Stimulating Factor.
7. The method of claims 1 or 2 wherein said selected medium contains Interleukin-6.
8. The method of claims 1 or 2 wherein said selected medium contains Mast Cell Growth Factor.
9. The method of claims 1 or 2 wherein said stem cells are separated on an affinity column.
10. The method of claims 1 or 2 wherein said stem cells are separated by flow cytometry.

11. The method of claims 1 or 2 wherein said separated stem cells are incubated in a petri dish.

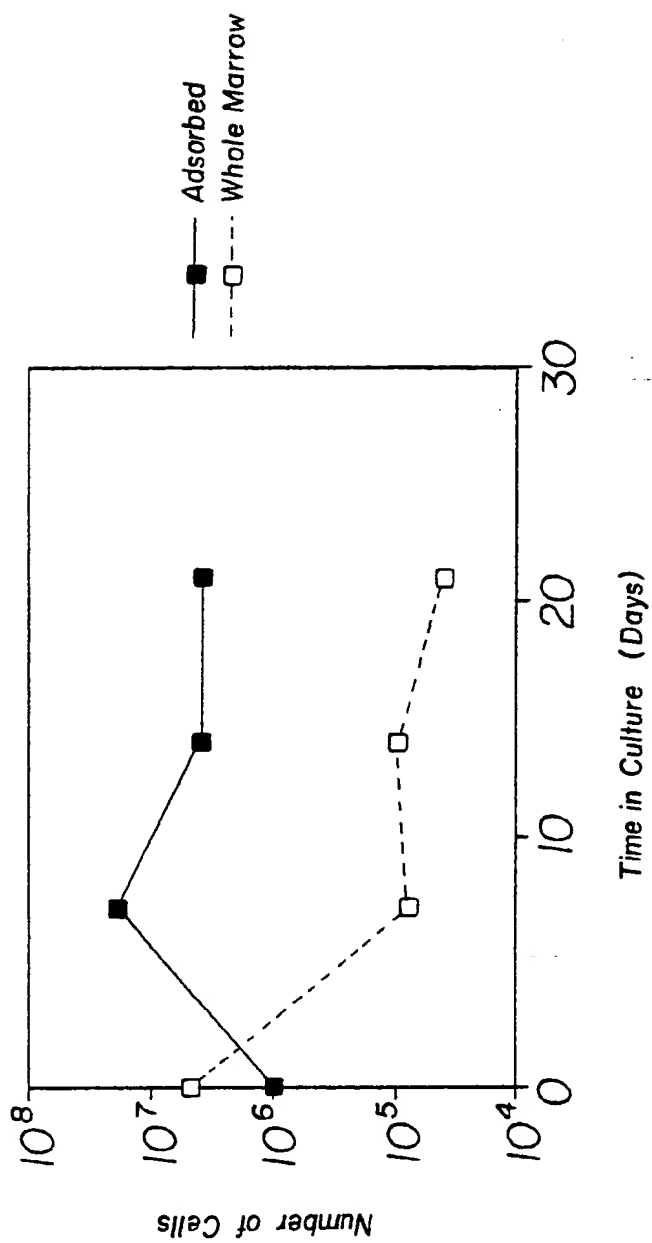
12. The method of claims 1 or 2 wherein said separated stem cells are incubated in a sterile bag.

13. The method of claims 1 or 2 wherein said separated stem cells are incubated in hollow fibers.

14. A method for selectively expanding stem cells, comprising:
(a) separating stem cells from other cells on an affinity column; and
(b) incubating the separated stem cells in a sterile bag with a medium containing Stem Cell Growth Factor, Interleukin-3, and Granulocyte-Macrophage Colony-Stimulating Factor.

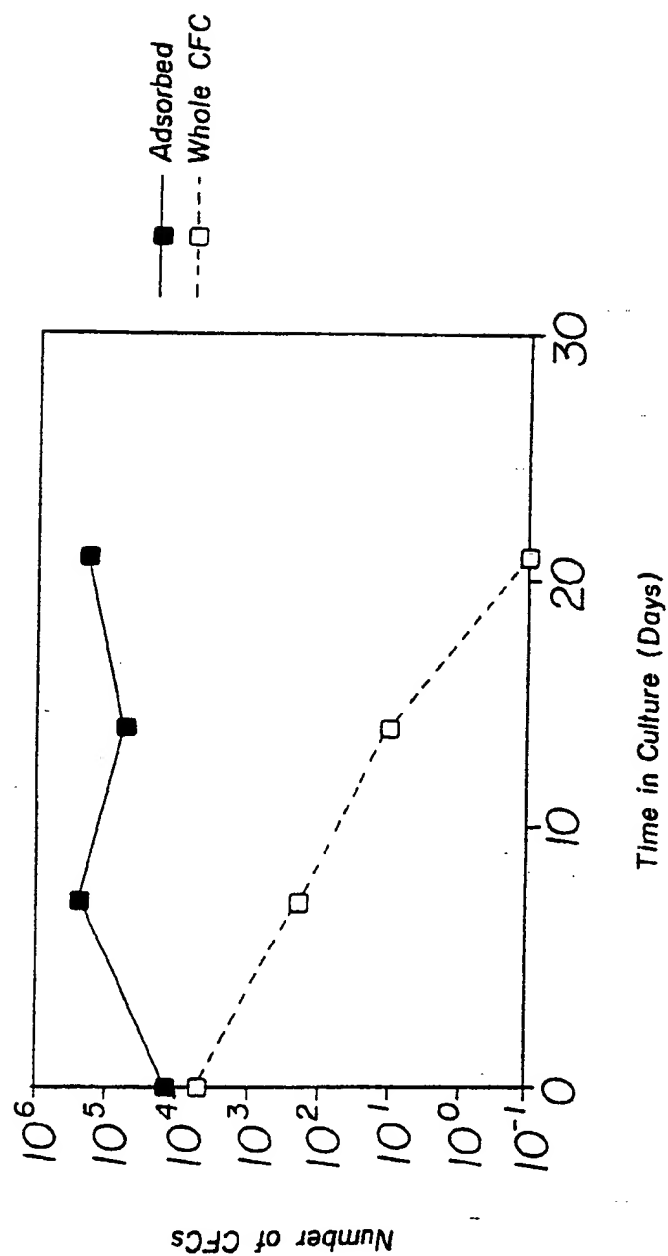
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FIG. 1



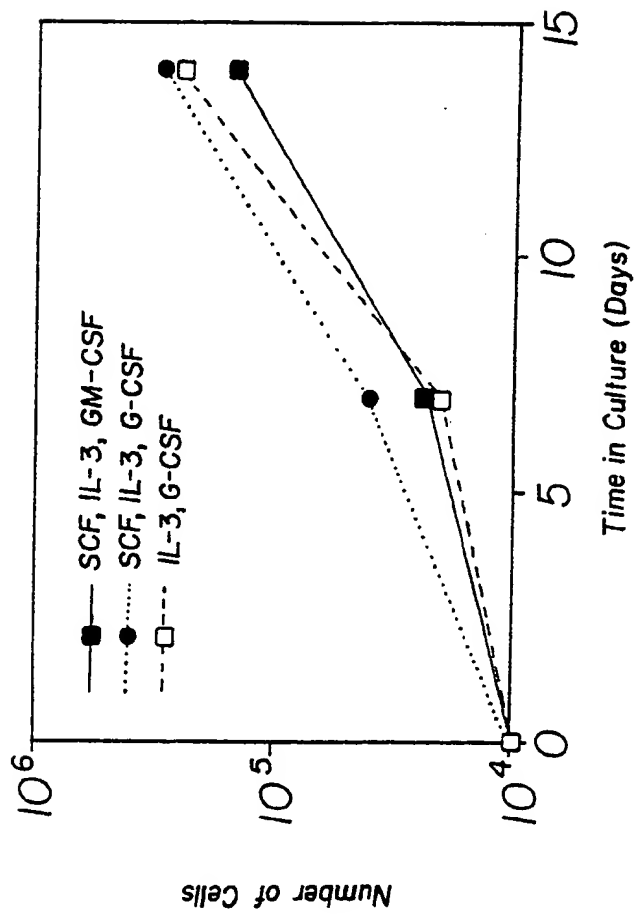
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FIG. 2



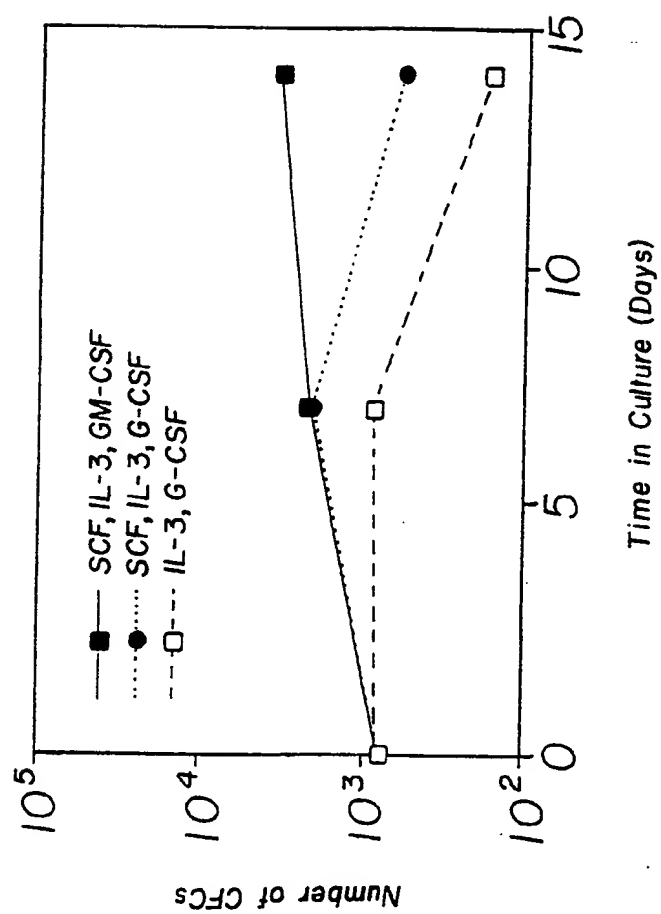
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FIG. 3



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FIG. 4



INTERNATIONAL SEARCH REPORT

PCT/US 92/09019

International Application No

| | | |
|--|---|---|
| I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ | | |
| According to International Patent Classification (IPC) or to both National Classification and IPC | | |
| Int.Cl. 5 C12N5/08 | | |
| II. FIELDS SEARCHED | | |
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| Classification System | Classification Symbols | |
| Int.Cl. 5 | C12N ; A61K | |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸ | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ | | |
| Category ¹⁰ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| X | EP,A,0 341 966 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 15 November 1989 see page 4, line 56 - page 5, line 12; claims --- | 1-14 |
| X | EP,A,0 451 611 (SYSTEMIX, INC.) 16 October 1991 see page 6, line 14 - line 23; claims --- | 1-14 |
| P,X | EP,A,0 455 482 (BECTON DICKINSON AND COMPANY) 6 November 1991 see page 5, line 29; claims 1-8,16,17,20,21 --- -/- | 1-14 |
| <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> | | |
| IV. CERTIFICATION | | |
| Date of the Actual Completion of the International Search | | Date of Mailing of this International Search Report |
| 28 JANUARY 1993 | | 18. 03. 93 |
| International Searching Authority EUROPEAN PATENT OFFICE | | Signature of Authorized Officer RYCKEBOSCH A.O. |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) | | |
|--|---|-----------------------|
| Category ° | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
| P,X | WO,A,9 201 039 (DANA-FARBER CANCER INSTITUTE ET AL.) 23 January 1992 see page 4, line 14 - page 5, line 28; claims 1-13; example 3 see page 9, line 7 - line 27 ----- | 1-14 |

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9209019
SA 66411

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 16/02/93. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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